Allergen Recognition Patterns in Walnut Allergy Are Age Dependent and Correlate with the Severity of Allergic Reactions

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Abstract: Background Walnut is an important elicitor of food allergy in children and adults with a high rate of severe reactions. Multicenter studies using a common clinical protocol and a comprehensive allergen are lacking. Objective To investigate potential correlations between molecular sensitization patterns and clinical characteristics of walnut-allergic patients. Methods A total of 91 walnut-allergic subjects and 24 tolerant controls from Switzerland, Germany, and Spain were included. Walnut allergy was established by food challenge in all but anaphylactic subjects. Specific IgE (sIgE) to walnut extract, rJug r 1 (2S albumin), rJug r 3 (nonspecific lipid transfer protein 1), nJug r 4 (11S globulin), rJug r 5 (PR-10 protein), 2 vicilin fractions, profiling, and cross-reactive carbohydrate determinant was determined by ImmunoCAP. A threshold of 0.10 kUA/L was used for positivity. Results Sensitivity of sIgE to walnut extract was 87% and increased to 96% for the sum of all walnut components. sIgE to walnut extract and all walnut components, except rJug r 5, was significantly higher in patients younger than 14 years at inclusion. Stratification by age at onset of walnut allergy led to similar results. All patients younger than 14 years had severe reactions, whereas 38% of patients 14 years or older were mild reactors. Severe reactors (n = 70) had higher sIgE levels than did mild reactors (n = 21) to walnut extract ($P < .0001$), rJug r 1 ($P < .0001$), nJug r 4 ($P = .0003$), and both vicilin fractions ($P < .0001$), but not to Jug r 3 and Jug r 5. Conclusions Sensitization to walnut storage proteins is acquired in childhood and correlates with severe reactions. sIgE levels to storage proteins Jug r 1 and Jug r 4 and vicilin fractions, but not to nonspecific lipid transfer protein and PR-10 proteins, correlate with systemic reactions to walnut.

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Allergen recognition patterns in walnut allergy are age dependent and correlate with the severity of allergic reactions

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Author contributions:

Barbara K. Ballmer-Weber has contributed to the conception and design of the study, clinical results, to the analysis of the results, the writing of the manuscript and has coordinated the clinical part of the study. Mariona Pascal, Joan Bartra, Lars Lange, Claudia Lang, Sunhild Gernert, Jaime Lozano-Blasco, Nora Gräni, Caroline Guillot contributed to design and conduct of the clinical part and reviewed the manuscript. Kay-Martin Hanschmann performed the statistical analysis. Bo Pontoppidan and Andrea Wangorsch contributed to the generation of the walnut allergen panel and Linda Tjäder to the generation of serological data. Jonas Lidholm and Stefan Vieths contributed to the conception and design of the study, data analysis and writing of the paper.

Author conflicts of interests:

B.K. Ballmer-Weber has received consultancy and lecture fees from Thermo Fisher Scientific; C. Lang received travel grants from Menarini Group, Novartis and ALK. C. Guillot received travel grants from ALK and Shire. J. Lidholm, B. Pontoppidan and L. Tjäder are employees of Thermo Fisher Scientific. L. Lange has received lecture fees from Thermo Fisher Scientific. S. Vieths has received travel support, grant funding, and honoraria from various organizations, but not from pharmaceutical industry or food industry. N. Gräni, S. Gernert, A. Wangorsch, K.M. Hanschmann, Mariona Pascal, Joan Bartra and Jaime Lozano-Blasco declare that they have no relevant conflicts of interest regarding this manuscript.

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Abstract

Background: Walnut is an important elicitor of food allergy in children and adults with a high rate of severe reactions. Multicenter studies using a common clinical protocol and a comprehensive allergen are lacking.

Objective: To investigate potential correlations between molecular sensitization patterns and clinical characteristics of walnut allergic patients.

Methods: 91 walnut allergic subjects and 24 tolerant controls from Switzerland, Germany and Spain were included. Walnut allergy was established by food challenge in all but anaphylactic subjects. sIgE to walnut extract, rJug_r_1 (2S albumin), rJug_r_3 (nsLTP1), nJug_r_4 (11S globulin), rJug_r_5 (PR-10 protein), two vicilin fractions, profilin and CCD was determined by ImmunoCAP. A threshold of 0.10 kU_A/L was used for positivity.

Results: Sensitivity of sIgE to walnut extract was 87% and increased to 96% for the sum of all walnut components. sIgE to walnut extract and all walnut components, except rJug_r_5, was significantly higher in patients below 14 years of age at inclusion. Stratification by age at onset of walnut allergy led to similar results. All patients <14 years had severe reactions whereas 38% of patients $\geq$14 years were mild reactors. Severe reactors (n=70) had higher sIgE levels than mild reactors (n=21) to walnut extract ($p<0.0001$), rJug_r_1 ($p<0.0001$), nJug_r_4 ($p=0.0003$) and both vicilin fractions ($p<0.0001$), but not to Jug_r_3 and Jug_r_5.

Conclusion: Sensitization to walnut storage proteins is acquired in childhood and correlates with severe reactions. sIgE levels to storage proteins Jug_r_1, Jug_r_4 and vicilin fractions, but not to nsLTP and PR-10 proteins, correlate with systemic reactions to walnut.

Highlights box:

1. What is already known about this topic?

   Walnut allergy is an important and prevalent tree nut allergy associated with severe allergic symptoms.

2. What does this article add to our knowledge?

   The study provides information on the relative importance of all known walnut allergens across Europe and evidence suggesting that that vicilins may be as important as 2S albumin in walnut allergy.

3. How does this study impact current management guidelines

   Patients with early onset walnut allergy have an elevated risk of severe reactions and should be given particular attention in the clinic.

Key words: walnut, food allergy, component resolved diagnosis, food challenge, age, severity, vicilin

Key words: Abstract: 249; Paper 3586

Abbreviations

CCD	cross-reactive carbohydrate determinant
DBPCFC	double-blind placebo-controlled food challenge
sIgE	specific IgE
SPT	skin prick test
CRD	component resolved diagnostics
PTP	prick to prick test

Introduction

Walnuts are seeds from the trees of Juglans genus, which consists of 24 different species. The *Juglans regia* species, the common or Persian walnut, is the most frequently consumed in Europe.

According to data from the Europrevall project, 3% of the adult European population is predicted to be sensitized to walnut, ranging from 0.1% in Iceland to 6 and 8% in Switzerland and Spain, respectively (1). Among children and adolescents with anaphylaxis to tree nuts, walnut belonged to the most important elicitors, accounting for 16% of the cases (2). According to telephone interviews with almost 5000 random households in the US, walnut was the most common offending tree nut, reported as a trigger in 60% of tree nut allergic individuals (3). In Chile, a cross-sectional questionnaire-based study indicated that allergy to walnut was the most prevalent food allergy in school aged children (4).

Threshold dose distribution data revealed that 3.1-4.1 mg walnut protein, corresponding to 1/180 of a walnut kernel, elicited an allergic reaction in 5% of a walnut allergic population (5). Despite of the emerging importance of walnut allergy, there is a scarcity of data on the importance of individual walnut allergens.

Allergens identified in walnut to date are the storage proteins Jug r 1 (2S albumin), Jug r 4 (11S globulin), Jug r 2 and Jug r 6 (vicilins) and the non-specific lipid transfer protein (nsLTP) Jug r 3. Moreover, walnut allergens with cross-reactivity to pollen are known; mainly a profilin, Jug r 7, and the recently described Bet v 1 homologous protein Jug r 5 (6-12). Here we present data of the most comprehensive study to date on molecular sensitization patterns to all known and officially accepted walnut allergens (www.allergen.org) in patients with confirmed walnut allergy, including children and adults, patients from Mediterranean and from birch pollen exposed regions, with a particular focus on severity and age of development of food allergy to walnut.

Design and Methods

Study design and subjects

Four allergy clinics in Switzerland (CH, Zurich and Lucerne), Germany (DE, Bonn) and Spain (ES, Barcelona) participated in this prospective multi-centre study. The clinical part of the study took place between 2013 and 2017. The study was approved by each centre's local ethical committee and all patients gave written informed consent before entering the study (KEK-ZH 2012-0519, EKNZ-2015-368; CEIC-2012/7085; AEKNO-2013310.).

In total, 115 individuals were included. All patients underwent the same clinical evaluation, including an extensive interview using a uniform questionnaire and blood sampling.

Final inclusion criteria for the group of walnut allergic patients (n=91) were a positive food challenge (double-blind placebo-controlled challenge (DBPCFC) or an open titrated food challenge (OFC)) or a convincing case history of anaphylaxis following ingestion of walnut. Sixty-one patients with a suggestive case history of a walnut allergy were included after agreeing to a food challenge. The remaining 30 patients were included on the basis of a case history of an anaphylactic reaction, defined as severe life-threatening reaction such as drop of blood pressure, severe bronchospasm or laryngeal edema within two hours after ingestion of walnut without performing a food challenge. Atopic controls were recruited from CH (n=5), DE (n=3) and ES (n=6) and 10 non-atopic controls from CH (n=5) and ES (n=5). The atopic controls had a history of pollen allergy with a positive SPT and/or positive sIgE test to relevant pollen allergens and were tolerant to walnut. The non-atopic controls had no history of pollen or food allergy and negative skin prick tests (SPT) and/or sIgE tests to a panel of food and inhalant allergens.

Food challenges

Open titrated food challenges and DBPCFCs were performed according to a common protocol and consisted of the patient ingesting increasing doses of walnuts (5mg, 10mg, 50mg, 100mg, 1.0g, 5.0g, 8.0g). A detailed description of the procedure is provided in this article's Online Repository at www.jaci-inpractice.org. For the purposes of the study, each patient was attributed to one of the following four clinical reaction groups: group 1: patients with isolated subjective oral symptoms under provocation (OAS); group 2: patients with an "extended OAS" under provocation (OAS with either blisters of the oral mucosa, mild rhinitis or conjunctivitis, slight swelling of the lips, mild tightness of the throat or mild dysphagia); group 3: objective systemic reactions under provocation; group 4: history of anaphylaxis.

Specific IgE antibody measurements

For all patients and controls, serum sIgE to walnut, rJug r 1, rJug r 3, nJug r 4, rJug r 5, high and low molecular weight walnut vicilin fractions, rPru p 3, rBet v 1, rBet v 2 and CCD was determined by ImmunoCAP. Additionally, sIgE to rPla a 3 was determined in the Spanish patients and controls. sIgE values $\geq$0.1 kU_A/L were considered positive. Purification of nJug r 4 and high and low molecular weight vicilin fractions from walnut, generation of rJug r 5 and of experimental ImmunoCAP tests is described in this article's Online Repository at www.jaci-inpractice.org.

Skin testing

Prick-to-prick tests (PTP) with native walnuts were performed in all walnut allergic patients and skin prick tests (SPT) with walnut extract (ALK-Abello, Madrid, Spain) in Swiss and Spanish patients.

Statistics

Differences between clinical reaction groups (mild vs. severe reactors) and between age groups (<14 years of age vs. ≥14 years of age) with respect to mean sIgE concentration were evaluated with Two-Sample Wilcoxon Test. For frequencies, Fisher's exact test was used.

A logistic regression model was applied with clinical severity (occurrence of systemic reactions) as dependent variable and sex, age at inclusion into study (younger / older than 14 years), age at onset of walnut allergy (younger / older than 14 years), geographical region (Switzerland and Germany versus Spain), and sensitization to walnut components (below/equal or above threshold 0.10 kU_A/L) as independent variables. Additionally, data was evaluated with univariate logistic regression models. The statistical analysis was performed with SAS®/STAT software, version 9.4, SAS System for Windows.

Results

Patients' characteristics and food challenges

Overall, 91 walnut allergic patients were enrolled, 31 adolescents and adults from CH (22 females (f), 9 males (m), age 34±12 years (y), range 15-68 y), 31 from DE (15 f, 9 m, age 6±3 y, range 2-15 y) and 29 from ES (15 f, 14 m, age 32±16 y, range 3-53 y). Fifteen patients were included on the basis of a positive DBPCFC (DBPCFC+ve), 46 patients on a positive titrated oral food challenge (OFC+ve) and 30 patients on a positive case history of walnut induced anaphylaxis. Furthermore, 14 atopic controls with pollen allergy and a history of walnut tolerance and 10 non-atopic controls were analysed. In 60 patients (68%), the onset of walnut allergy was before their 14th birthday.

Sixteen challenge positive patients (18%) reported an isolated OAS under provocation and five an extended OAS with mild additional symptoms of the lips or throat, or rhinoconjunctivits, and 40 patients developed a systemic reaction, 75% with skin symptoms such as flush, urticaria, angioedema, 33% with respiratory symptoms, 23% with gastrointestinal symptoms and 8% with laryngeal involvement. Table E1 summarizes the clinical reactivity of all included walnut allergic patients. Allergic reactions to the following tree nuts were reported by the 91 walnut allergic subjects: pecan nut (9%), hazelnut (44%), almond (18%), brazil nut (13%), cashew (11%), pistachio (13%) and macadamia (8%).

Sensitization to walnut extract and individual allergens

PTP was positive in all tested walnut allergics from Spain and Germany (n=51) and in 13 out of 27 Swiss patients (overall sensitivity 82%) whereas SPT with walnut extract was positive in 25/29 Spanish patients and in 17/30 Swiss patients (overall sensitivity 71%). For serum sIgE testing the threshold for positivity was set to 0.10 kU_A/L. This cut-off was selected on the basis of a ROC analysis for sIgE to walnut extract, providing a sensitivity of 98.6% (95% Confidence Interval (CI): 92.3%-100%) and a specificity of 52.4% (95% CI: 29.8%-74.3%) for diagnosing patients with systemic reactions to walnut (groups 3 and 4).

Considering the entire walnut allergic population, the sensitivity of sIgE to walnut extract was 87%, whereas 96% were sensitised to at least one walnut component. In patients with mild symptoms (group 1 and 2) sensitivity of sIgE measurement to walnut extract was particularly compromised and as low as 48%. Eight of eleven patients with mild symptoms but no sIgE to walnut extract were sensitised to the Bet v 1 homologous protein rJug r 5. Thus, only rJug r 5 accounted for the increased sensitivity of component testing compared to the whole walnut extract. Four patients, three with mild symptoms and one with anaphylaxis, tested negative to walnut extract and did not recognize any of the included components. In total, 53% of walnut allergic subjects were sensitized to rJug r 1, 53% to rJug r 3, 48% to nJug r 4, 52% to rJug r 5, 57% to nJug r vicilin-H, 73% to nJug r vicilin-L, 17% to profilin (rBet v 2) and 19% to CCD. The concentration of IgE to the single walnut allergens is summarized in Figure 1.

Sensitization profile in atopic and nonatopic controls

All nonatopic controls tested completely negative to walnut extract and allergens. Five Swiss and one German atopic controls were sensitized to rJug r 5 and all presented relatively high levels of sIgE to rBet v 1. Three Spanish and two German atopic controls were sensitised to rJug r 3, four of them to rPru p 3 and one to rPla a 3 at higher concentrations. Sensitisation to rJug r 1 (3/3 patients) and nJug r 4 (1/3 patients) was only observed in the three German pediatric atopic controls. Sensitisation rates to nJug r Vicilin-H and nJug r Vicilin-L among the atopic controls were 43% and 36%, respectively. Since the number of controls was limited, specificity in relation to the controls was not calculated.

Geographic differences in sensitization pattern

Figure 2 summarizes the sensitization pattern of patients from the three geographic regions to walnut extract and the single allergen components. Particularly prominent differences were observed for sensitization to rJug r 3, which was higher in ES than in CH and DE and for rJug r 5, which was highest in CH and DE. In DE much higher sensitisation rates to rJug r 1 and

nJug r 4 were observed than in CH and ES, but this finding was confounded by the fact that in DE, a purely pediatric population was recruited.

Age dependency of the sensitization pattern to walnut allergens

Mean concentrations of sIgE to walnut extract and all walnut components except rJug r 5 were significantly higher in walnut allergic patients below 14 years of age than in those above that age at inclusion into the study. Also, if stratified by the age of walnut allergy onset, patients who acquired walnut allergy before the age of 14 had significantly higher sIgE concentrations to walnut extract and all walnut allergens except for rJug r 3 ($p< 0.0001$ for rJug r 1, nJug r 4, nJug r vicilin-H and nJug r vicilin-L, $p<0.01$ for walnut extract, $p<0.05$ for rJug r 5) than those with later onset (Table 1). Among the late onset reactors, only three subjects were sensitised to rJug r 1 (11%), two to nJug r 4 (7%) and six to nJug r vicilin-H (21%), whereas sensitisation rates to these storage proteins were significantly higher in the early onset allergics (75%, 70% and 77%, respectively, Figure 3), supporting the view that sensitisation to these storage proteins is primarily acquired during childhood.

Of the early onset allergics, 90% belonged to the severe reactors (group 3/4) whereas 46% of the late onset allergics belonged to the mild reactors (group 1/2). Thus, age at onset of allergy to walnut (<14 years vs ≥14 years) significantly correlated with the severity of the clinical reaction ($p=0.0002$; Fisher's exact test). If stratified by age at inclusion into the study, similar results were observed ($p<0.0001$; Fisher's exact test).

Are there any predictive markers for the severity of the clinical reaction?

Patients of clinical reaction groups 3/4 (severe reactors: n=70; systemic reaction under challenge or anaphylaxis) had significantly higher concentrations of sIgE to walnut extract ($p<0.0001$), rJug r 1 ($p<0.0001$), nJug r 4 ($p<0.0001$), nJug r vicilin-L ($p=0.0001$), and nJug r vicilin-H ($p<0.0001$) than patients of clinical reaction groups 1/2 (mild reactors: n=21; symptoms restricted to the oral cavity and mild symptoms of lip, throat or rhinoconjunctival

291 mucosa under challenge). No significant differences between these groups were found for sIgE
292 to rJug r 3 and rJug r 5 (Table 2). Nevertheless, when stratified for geographic regions (CH/DE
293 versus ES), significant differences between mild and severe reactors for sIgE concentrations
294 to walnut extract, rJug r 1, nJug r 4, nJug r vicilin-L and nJug r vicilin-H were found among the
295 Swiss and German walnut allergic patients but not among the Spanish patients. Thus, the level
296 of sensitisation to all analysed walnut allergens did not differ between mild and severe reactors
297 from Spain.
298 Sensitisation to rJug r 1 and nJug r 4 was only observed among the severe reactors (Figure
299 4). Furthermore, the sensitisation rate to nJug r vicilin-H and vicilin-L was much higher in the
300 severe reactors than in the mild reactors (71% vs 10% and 84% vs 33%, respectively).
301 Symptom severity (group 3/4, severe reactors vs group 1/2, mild reactors) was highly
302 significantly ($p<0.0001$; Fisher's exact test) associated with sensitisation to walnut extract, rJug
303 r 1, nJug r 4, nJug r vicilin-L and nJug r vicilin-H but not with sensitisation to rJug r 3 and rJug
304 r 5. Results were consistent when applying a cut-off level for positivity of 0.35 rather than 0.10
305 kU_A/L.
306 Logistic regression revealed a significant influence of sensitization to walnut extract and to
307 both vicilin fractions, as well as the age of onset of walnut allergy, on the severity of the
308 clinical reaction. However, since all patients included into the study at an age <14 years and
309 all patients sensitized to rJug r 1 belonged to the severe reactors, the odds ratio for
310 developing a severe reaction could not be calculated (not determinable). In the logistic
311 regression analysis, gender, geographic region and sensitisation to rJug r 3, nJug r 4 and
312 rJug r 5, all showed no influence on clinical severity ($p>0.05$).
313 The risk of developing a systemic reaction was significantly higher for walnut extract
314 sensitized patients (odds ratio (OR): 76.9; 95% CI: 8.7-500, $p<0.0001$), for nJug r vicilin-L
315 and vicilin-H sensitised patients (OR: 10.5 and 23.3, CI: 3.5-32.3 and 5.0-111, respectively,
316 $p<0.0001$) and for patients with an onset of walnut allergy before their 14^{th} birthday (OR:

317 7.655, CI: 2.487-23.563; p=0.0004) than for patients not sensitized to walnut extract, neither
318 of the vicilin fractions or acquisition of walnut allergy after their 14th birthday.
319
320

Discussion

To our knowledge, this study represents the most comprehensive CRD analysis done in walnut allergic children and adults so far. We quantified specific IgE concentrations to walnut allergens in 91 European walnut-allergic pediatric and adult individuals recruited in three countries, Germany, Switzerland and Spain, as well as in 24 walnut tolerant controls.

For serological analysis, a cut-off of 0.10 kU_A/L was used for sIgE positivity, but the majority of results, in particular on age dependency and severity, remained stable when the analyses were repeated with a cut-off of 0.35 kU_A/L.

Specific IgE to walnut extract showed a sensitivity of 87% which increased to 96% when sIgE to the Bet v 1 homologue Jug r 5 was taken into consideration. However, at the same time this led to a reduction of diagnostic specificity as six of the 14 atopic controls (43%) also showed positive test results to Jug r 5. As the size of our atopic and non atopic control groups were limited, an extended statistical analysis of our patient group data in comparison to the controls was not performed. However, the data appear to be in accordance with other CRD studies in plant food allergy (13-15), showing very high specificity in comparison to non allergic controls and negative effects on specificity caused by cross-reactivity of sIgE to Bet v 1 with PR-10 allergens from foods in pollen allergic patients without food allergy. Notably, we found little effect on diagnostic performance by sensitization to profilin and CCD, as the sensitization rates among the walnut allergic patients were below 20% and only 2 and 3 atopic controls were positive to these allergen components, respectively.

Sensitivity of PTP and SPT with native walnuts and the commercial walnut extract, respectively, was particularly affected in the CH population (48% and 57%), which can be most likely explained by a low content of Bet v 1 homologous proteins in tree nuts and a consequently low representation in the skin prick test extract.

Our data analysis revealed statistically significant differences between patients with mild symptoms (groups 1 and 2) and more severe / systemic symptoms (groups 3 and 4) on the one hand, and between patients below versus above 14 years of age on the other hand.

Geographic differences observed in our study need to be considered in view of the fact that the patient populations recruited in the different study centers were not fully comparable in terms of age. In Spain, adults/adolescents as well as children were included; in Switzerland the youngest patient was 15 years-old at inclusion, whereas in Germany only children up to 15 years of age were included, the majority being below 10. Keeping in mind these differences, we found the highest sensitization rate to the nsLTP Jug r 3 in Spain, whereas sensitization to the Bet v 1 homologue Jug r 5 was much more common in Switzerland and Germany. This observation is in full accordance with other studies describing a predominance of nsLTP sensitization in plant food allergy in southern Europe, as well as a dominating role of Bet v 1 related food allergens in birch endemic regions, such as Switzerland, Germany and Scandinavia (reviewed in 16-17). Moreover, higher sensitization rates to the storage proteins Jug r 1 (2S albumin) and Jug r 4 (11S globulin) were found in the German patient population. This finding reflects a more prominent role of the storage proteins in paediatric subjects with primary sensitization to walnuts.

Concerning age dependency, all patients <14 years of age had severe reactions (group 3/4), as compared to 62% of patients $\geq$14 years of age. In regard to serology, we found significantly higher mean levels of sIgE to walnut extract and Jug r 1, Jug r 3, Jug r 4, and the two vicilin preparations in patients below 14 years of age at inclusion versus patients above 14 years. When stratified for age of allergy onset, similar results were obtained, except that the difference was not significant for Jug r 3 (p=0.0985), but turned significant for Jug r 5 (p=0.0368) (Table 1). The frequency of detectable sIgE (regardless of level) was also different between late and early onset reactors, with sensitization to Jug r 1, Jug r 4, Jug r 5 and the vicilin preparations

being significantly more frequent in early onset allergics (Figure 3).

Taken together, our data on age dependency of sensitization patterns are comparable to findings obtained in peanut allergic patients recruited within the EuroPrevall study (18).

We also analysed a potential correlation of sIgE levels with severity of clinical reactions to walnut. Mean sIgE concentrations to walnut extract and all walnut allergens except Jug r 3 and Jug r 5 were significantly higher in clinical reaction groups 3/4 than in groups 1/2. Similar to reports in peanut allergy (18-20) and hazelnut allergy (21), this result supports the view that severe reactions to legume seeds and nuts are predominantly caused by sensitization to storage proteins rather than by pollen related allergens such as Bet v 1 homologues, or nsLTP. A plausible explanation for this phenomenon could be the relatively low content of Bet v 1 related proteins and nsLTP in such high protein containing foods, leading to a lower allergen intake in comparison to storage proteins which are present in much higher amounts.

Interestingly, no significant differences in sIgE concentrations between symptom groups 3/4 versus groups 1/2 were detected in the Spanish patients. While it is clear that this phenomenon cannot be explained by clinically insignificant sIgE binding to cross-reactive components such as PR-10 allergens, profilin, or nsLTP, it is difficult to establish a plausible hypothesis. We speculate that the more complex allergen environment in Spain in comparison to Switzerland and Germany may play a role, but a separate study will be required to confirm this finding and address potential reasons.

The two vicilin preparations used in this study showed a high frequency of sIgE binding (56% and 71% for vicilin-H and vicilin-L, respectively). These rates are the highest of all purified allergen preparations used in this study, suggesting that Jug r 2 and/or Jug r 6 may become important additions to the panel of allergens used in the diagnosis of walnut allergy.

In summary, our study showed that the sensitivity of sIgE testing in walnut allergy can be increased by component resolved diagnosis and that this increase is mainly related to the Bet v 1 related walnut allergen, Jug r 5. Our results support the view that sensitization to walnut

storage proteins is usually acquired in childhood and is associated with severe allergic reactions. IgE to storage proteins Jug r 1, Jug r 4 and vicilin fractions, but not to nsLTP and PR-10 proteins, is significantly associated with systemic reactions to walnut. Provided that these findings are confirmed by further studies in well-characterised patients with food allergy to walnut, sIgE to these storage proteins may come to serve as a marker for an increased risk of severe food allergy to walnut.

Legends to figures

Figure 1:
IgE concentration to walnut extract and to the different walnut allergen components in all walnut allergic patients and in patients from CH, ES, DE. IgE to profilin and CCD is not included, but described in the text. Numbers in brackets indicate the number of observations below the 0.1 kU_A/L cut-off. Horizontal bars: median; dotted line: cut-off.

Figure 2:
Percentage of patients with sensitization to walnut extract and to the different components in walnut allergic patients from CH, ES, DE.

Figure 3:
Percentage of patients with sensitization to walnut extract and to the different components in subjects with onset of walnut allergy before their 14th birthday and beyond the age of 14 years.

Figure 4:
Percentage of patients with sensitization to walnut extract and to the different components in walnut allergic patients with severe reactions (group 3/4, systemic reaction under provocation or anaphylaxis by history) and mild reactors (group 1/2, with mild symptoms under provocation).

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